THE USE OF VIRULENT SALT SOLUTION AS A VIRUS IN MANUFACTURING HOG CHOLERA SERUM.*

W. S. ROBBINS.

(From the Michigan Agricultural College, Experiment Station, East Lansing, Michigan.)

Soon after the demonstration at Ames, Iowa, in 1908, to show methods of manufacture and value of Dorset-Niles serum toward controlling hog cholera, many of the agricultural experiment stations became interested and have since put forth vigorous efforts toward controlling this disease with hog cholera serum.

Although carrying many unsolved problems, hyperimmune serum has been efficient in preventing cholera and no doubt has been of great aid in controlling this disease. The control of cholera in any section depends to a great extent upon the expense involved, and, in case the serum is to be used, its price must necessarily be considered. Therefore, considering the cost of producing serum, there seems to be a demand for research which will result in a reduction of the expense.

In manufacturing serum by the regular Dorset-Niles methods, the cholera pig is sacrificed merely for its blood, and this virus blood, unless injected by the intravenous method, is sufficient only to hyperimmunize one pig of weight equal to that of the virus pig. Thus, we can readily see that reducing the cost of manufacturing serum by this method depends to a great extent upon reducing the cost of a virus that will prove efficient in hyperimmunizing.

Taking up the idea of Dr. Craig, of the Indiana Agricultural Experiment Station, this experiment was undertaken as a problem of practical value, with the purpose of reducing the cost of serum by using salt solution as a virus when passed through the abdominal cavity of virus pigs. It was our desire to determine the value of this saline solution as a virus and, if possible, to advance methods of using it that would prove efficient and practical.

Since an accurate method of standardizing serum or virus is

* Received for publication March 5, 1913.

lacking, and the virulence of virus varies according to its source, and the potency of serum varies according to virulence of virus used, amount of virus injected, and condition of pig used for hyperimmune before and after injecting, I found that it required numerous and careful tests to establish our results, each being run parallel with the Dorset-Niles subcutaneous methods as a check.

In this experiment, I used 0.75 per cent to 0.85 per cent salt solution. The solution was sterilized and kept so in cotton-plugged flasks. Just before using, it was heated to about 37.5° C. It was injected at this temperature by means of a sterile aspirator apparatus into the abdominal cavities of virus pigs, in varying amounts, and allowed to remain various lengths of time so that we might determine just what time would prove most satisfactory and the approximate amount to inject. That portion of the solution remaining unabsorbed was removed from the peritoneal cavity by means of a sterile pipette or a small casserole immediately after killing the pig.

In this work, I have injected 56 virus pigs with salt solution. The injection varied in amount from 20 c.c. to 45 c.c. per pound of body weight and varied in time remaining in abdominal cavity from 3 hours to 14 hours as shown in Table 1 of virus pigs.

With the virulent salt solution secured from abdominal cavities of virus pigs, I have hyperimmunized 43 pigs, injecting the virus subcutaneously at the rate of from 10 c.c. to 20 c.c. per pound of body weight as shown in the following table of hyperimmunes (Table 2).

It is generally admitted by serum manufacturers that the amount of virus blood secured from virus pigs averages about 10 c.c. per pound of body weight. Accepting this as a standard, I have endeavored to calculate the percentage of increase of virulent material secured when salt solution was injected intra-abdominally as follows (Table 3).

How does salt solution gain virulence when injected into abdominal cavity of virus pigs? It is impossible at present to state just how salt solution gains its virulence while in the abdominal cavity, and knowing very little of the ultramicroscopic virus causing hog cholera, it is impossible to determine just how virulent

this solution may become. The virulence no doubt varies greatly with different pigs even though autopsies correspond. As far as

TABLE 1.

EXPERIMENTAL VIRUS PIGS INJECTED INTRA-ABDOMINALLY WITH PHYSIOLOGICAL SALT SOLUTION.

Pig No.	Wt. Lbs.	Amount of NaCl Solution Injected	No. c.c. per Lb.	Time Remaining in Abdominal Cavity	Amount of Virus NaCl Recovered	Percentage Recovered	Average Percentage Recovered	Amount of Virus Blood Secured	Approximate Increase of Virus Blood Due to NaCl Injected
267.....	79	2,000 c.c.	25	3 hrs.	1,250 c.c.	62.5	....	875 c.c.	85 c.c.
264.....	110	2,750 c.c.	25	3 hrs.	1,975 c.c.	71.8	67.1	1,250 c.c.	150 c.c.
270.....	121	3,025 c.c.	25	4 hrs.	1,500 c.c.	49.5	....	1,550 c.c.	340 c.c.
272.....	81	2,025 c.c.	25	4 hrs.	1,550 c.c.	76.5	....	1,550 c.c.	740 c.c.
276.....	100	2,500 c.c.	25	4 hrs.	1,550 c.c.	62.0	....	1,400 c.c.	400 c.c.
278.....	78	1,950 c.c.	25	4 hrs.	1,100 c.c.	56.4	....	1,000 c.c.	320 c.c.
277.....	122	3,000 c.c.	25	4 hrs.	1,300 c.c.	43.3	....	850 c.c.	.....
461.....	71	1,875 c.c.	25	4 hrs.	1,000 c.c.	53.3	56.8	900 c.c.	190 c.c.
433.....	80	1,600 c.c.	20	4 hrs.	650 c.c.	40.6	40.6	1,100 c.c.	300 c.c.
284.....	94	2,820 c.c.	30	4 hrs.	1,400 c.c.	49.6	....	1,200 c.c.	260 c.c.
479.....	68	2,040 c.c.	30	4 hrs.	700 c.c.	34.3	41.9	600 c.c.	.....
476.....	90	4,050 c.c.	45	4 hrs.	2,200 c.c.	54.8	54.8	1,100 c.c.	200 c.c.
280.....	82	2,460 c.c.	30	5 hrs.	1,500 c.c.	65.0	....	1,100 c.c.	280 c.c.
281.....	134	4,020 c.c.	30	5 hrs.	2,600 c.c.	67.1	....	1,600 c.c.	260 c.c.
282.....	124	3,750 c.c.	30	5 hrs.	2,200 c.c.	58.6	....	1,000 c.c.	.....
283.....	108	3,300 c.c.	30	5 hrs.	2,600 c.c.	78.7	....	1,650 c.c.	560 c.c.
285.....	128	3,840 c.c.	30	5 hrs.	2,200 c.c.	57.0	....	1,650 c.c.	360 c.c.
290.....	95	2,700 c.c.	30	5 hrs.	1,400 c.c.	51.1	....	1,350 c.c.	400 c.c.
291.....	96	2,880 c.c.	30	5 hrs.	2,000 c.c.	62.5	....	1,100 c.c.	140 c.c.
292.....	83	2,490 c.c.	30	5 hrs.	1,250 c.c.	52.2	....	1,200 c.c.	270 c.c.
422.....	100	3,000 c.c.	30	5 hrs.	1,800 c.c.	60.0	....	1,100 c.c.	100 c.c.
474.....	76	2,300 c.c.	30	5 hrs.	600 c.c.	26.0	57.8	1,000 c.c.	240 c.c.
434.....	88	3,000 c.c.	35	5 hrs.	2,000 c.c.	66.6	....	1,100 c.c.	220 c.c.
493.....	90	3,250 c.c.	35	5 hrs.	1,500 c.c.	46.1	46.1	1,200 c.c.	300 c.c.
443.....	75	3,000 c.c.	40	5 hrs.	1,200 c.c.	40.0	....	1,100 c.c.	350 c.c.
454.....	53	2,250 c.c.	40	5 hrs.	1,400 c.c.	63.0	51.5	1,200 c.c.	670 c.c.
406.....	60	2,700 c.c.	45	5 hrs.	1,300 c.c.	48.1	....	1,100 c.c.	500 c.c.
485.....	100	4,500 c.c.	45	5 hrs.	2,700 c.c.	60.0	54.5	1,400 c.c.	400 c.c.
403.....	117	3,000 c.c.	25	6 hrs.	2,000 c.c.	66.6	....	1,600 c.c.	430 c.c.
384.....	88	3,000 c.c.	30	6 hrs.	2,000 c.c.	66.6	....	1,100 c.c.	220 c.c.
431.....	100	3,000 c.c.	30	6 hrs.	1,000 c.c.	33.4	50.0	1,700 c.c.	700 c.c.
341.....	85	2,500 c.c.	33	6 hrs.	900 c.c.	36.0	....	1,000 c.c.	150 c.c.
414.....	85	3,000 c.c.	36	6 hrs.	1,600 c.c.	53.3	....	1,000 c.c.	150 c.c.
432.....	72	2,500 c.c.	36	6 hrs.	1,200 c.c.	48.0	50.5	1,000 c.c.	280 c.c.
438.....	73	3,000 c.c.	40	6 hrs.	2,000 c.c.	66.6	....	1,400 c.c.	670 c.c.
423*a*	77	3,200 c.c.	40	6 hrs.	1,700 c.c.	53.1	59.5	1,000 c.c.	230 c.c.
422*b*	60	2,500 c.c.	42	6 hrs.	1,400 c.c.	56.0	56.0	1,100 c.c.	500 c.c.
461*b*	70	3,150 c.c.	45	6 hrs.	1,200 c.c.	38.0	38.0	1,220 c.c.	420 c.c.
415.....	104	3,000 c.c.	27	6½ hrs.	2,000 c.c.	66.6	66.6	1,700 c.c.	660 c.c.
407.....	107	2,500 c.c.	23	7 hrs.	1,000 c.c.	40.0	40.0	1,200 c.c.	130 c.c.
286.....	96	3,000 c.c.	30	7 hrs.	1,800 c.c.	60.0	....	1,200 c.c.	240 c.c.
418*a*	88	2,700 c.c.	30	7 hrs.	650 c.c.	24.0	42.0	1,200 c.c.	320 c.c.
423*b*	93	3,000 c.c.	31	7 hrs.	1,200 c.c.	40.0	40.0	1,200 c.c.	270 c.c.
346.....	100	3,400 c.c.	34	7 hrs.	1,800 c.c.	52.9	52.9	1,600 c.c.	600 c.c.
435.....	78	3,000 c.c.	38	7 hrs.	1,300 c.c.	43.3	43.3	1,000 c.c.	220 c.c.
431.....	70½	3,000 c.c.	42	7 hrs.	2,100 c.c.	70.0	70.0	1,700 c.c.	1,000 c.c.
404.....	143	3,000 c.c.	20	7½ hrs.	2,000 c.c.	66.6	66.6	2,400 c.c.	970 c.c.
406.....	103	2,500 c.c.	24	7½ hrs.	1,000 c.c.	40.0	40.0	1,100 c.c.	70 c.c.
289.....	98	3,000 c.c.	30	7½ hrs.	2,100 c.c.	70.0	70.0	1,200 c.c.	220 c.c.
380.....	77	3,000 c.c.	39	7½ hrs.	2,200 c.c.	73.3	73.3	1,200 c.c.	430 c.c.
428.....	68	2,680 c.c.	40	7½ hrs.	2,000 c.c.	74.6	74.6	1,000 c.c.	320 c.c.
402.....	132	3,000 c.c.	22	8 hrs.	2,000 c.c.	66.6	66.6	1,600 c.c.	280 c.c.
417.....	92	2,700 c.c.	30	8 hrs.	None	00.0	00.0	1,000 c.c.	80 c.c.
411.....	84	2,000 c.c.	24	9 hrs.	900 c.c.	45.0	45.0	700 c.c.	.....
408.....	93	2,500 c.c.	26	11 hrs.	1,200 c.c.	48.0	48.0	1,250 c.c.	320 c.c.
418*b*...	88	2,000 c.c.	25	14 hrs.	None	00.0	00.0	1,200 c.c.	320 c.c.

our methods of standardizing virus go, we fail to see a decrease in virulence of virus blood due to injecting salt solution, although

TABLE 2.

EXPERIMENTAL PIGS, HYPERIMMUNIZED WITH VIRUS SALT SOLUTION.

HYPER-IMMUNE NO.	WEIGHT IN POUNDS	AMOUNT OF VIRUS NaCl INJECTED TO HYPERIMMUNIZE	NO. C.C. INJECTED PER LB. OF BODY WEIGHT.	DATA ON VIRUS NaCl USED FOR HYPER-IMMUNIZATION			AMOUNT OF VIRUS NaCl INJECTED TO REHYPER-IMMUNIZE	NO. C.C. PER LB. INJECTED TO REHYPERIMMUNIZE	DATA ON VIRUS NaCl USED FOR REHYPER-IMMUNIZATION			TOTAL BLEEDINGS OF HYPER-IMMUNES	TOTAL AMOUNT OF SERUM SECURED	RESULT OF SERUM TEST	
				TIME VIRUS REMAINED IN ABDOMINAL CAVITY OF VIRUS PIGS	NO. C.C. PER LB. OF NaCl SOL. INJ. INTO ABDOMINAL CAVITY OF VIRUS PIGS.	PER CENT OF NaCl SOL. RECOVERED FROM VIRUS PIGS			TIME VIRUS NaCl REMAINED IN ABDOMINAL CAVITY OF VIRUS PIGS	PERCENT OF INJECTED NaCl SOL. RECOVERED FROM VIRUS PIGS	NO. C.C. NaCl PER LB. INJ. INTO ABDOMINAL CAVITY OF VIRUS PIGS			NO. C.C. PROTECTED TEST PIGS AGAINST 2C.C. VIRUS BLOOD	NO. C C. FAILED TO PROTECT TEST PIGS
246.....	84	630 c.c. NaCl 420 c.c. V.B.	7½ 5	3 hrs.	25	62.5	.....	....	.....	....	....	4	2,700 c.c.	35	10–15–20 25–30
247.....	90	450 c.c. NaCl 450 c.c. V.B.	5 5	3 hrs.	25	62.5	.....	....	.....	....	....	4	2,900 c.c.	.....	10–15–20–25 30–35
250.....	90	900 c.c. NaCl	10	3 hrs.	25	71.8	.....	....	.....	....	....	4	3,000 c.c.	35	10–15–20 25–30
329.....	65	650 c.c. NaCl	10	4 hrs.	20	40.6	.....	....	.....	....	....	4	2,890 c.c.	20	10–15
251.....	95	712 c.c. NaCl 475 c.c. V.B.	12½	4 hrs.	25	76.5	.....	....	.....	....	....	4	2,900 c.c.	10–15–20	.....
252.....	72	360 c.c. NaCl 360 c.c. V.B.	5 5	4 hrs.	25	76.5	.....	....	.....	....	....	4	2,200 c.c.	15–20	10
254.....	118	1,475 c.c. NaCl	12½	4 hrs.	25	49.5	.....	....	.....	....	....	4	3,400 c.c.	10–15–20	.....
256.....	106	1,060 c.c. NaCl	10	4 hrs.	25	62.0	.....	....	.....	....	....	4	3,300 c.c.	15–20	10
257.....	120	610 c.c. NaCl 610 c.c. V.B.	5 5	4 hrs.	25	56.4	.....	....	.....	....	....	4	4,000 c.c.	10–15–20	.....
258.....	80	400 c.c. NaCl 400 c.c. V.B.	5 5	4 hrs.	25	56.4	.....	....	.....	....	....	4	2,700 c.c.	10–20	15
260.....	128	1,300 c.c. NaCl	10	4 hrs.	25	43.3	.....	....	.....	....	....	4	4,400 c.c.	10–15–20	.....
330.....	80	800 c.c. NaCl	10	4 hrs.	25	53.3	.....	....	.....	....	....	4	3,000 c.c.	20	10–15
323.....	100	700 c.c. NaCl 300 c.c. V.B.	7 3	4 hrs.	30	34.3	.....	....	.....	....	....	4	2,700 c.c.	20–25	10–15
267.....	135	1,400 c.c. NaCl	10	4 hrs.	30	49.6	.....	....	.....	....	....	4	4,500 c.c.	10–15–20	.....
341.....	110	1,100 c.c. NaCl	10	4 hrs.	45	54.8	.....	....	.....	....	....	4	3,695 c.c.	20	10–15
269.....	105	1,575 c.c. NaCl	15	5 hrs.	30	67.1	.....	....	.....	....	....	4	3,800 c.c.	15–20	10
268.....	135	2,025 c.c. NaCl	15	5 hrs.	30	72.9	.....	....	.....	....	....	4	3,150 c.c.	10–15–20	.....
270.....	120	1,800 c.c. NaCl	15	5 hrs.	30	70.0	900 c.c.	7½	9 hrs.	45.0	24	7	4,470 c.c.	10–15–20	.....
262.....	160	2,400 c.c. NaCl	15	5 hrs.	30	57.3	.....	....	.....	....	....	4	4,500 c.c.	10–15–20	.....
263.....	150	1,900 c.c. NaCl	15	5 hrs.	30	57.3	.....	....	.....	....	....	4	4,600 c.c.	15–20	10

TABLE 2—*Continued*

290.....	120	600 c.c. NaCl 600 c.c. V.B.	5 5	5 hrs.	30	52.2	.....	....	.....	....	....	4	3,560 c.c.	10–15–20	.....
291.....	120	600 c.c. NaCl 600 c.c. V.B.	5 5	5 hrs.	30	52.2	.....	....	.....	....	....	4	3,500 c.c.	10–15–20	.....
271.....	120	1,440 c.c. NaCl	12	5 hrs.	30	70.2	.....	....	.....	....	....	4	3,500 c.c.	10–15–20	.....
322.....	90	600 c.c. NaCl 600 c.c. V.B.	10	5 hrs.	30	26.0	.....	....	.....	....	....	4	3,000 c.c.	10–15–20	.....
345.....	120	1,200 c.c. NaCl	10	5 hrs.	35	46.1	.....	....	.....	....	....	4	3,320 c.c.	.....	.....
320.....	100	1,000 c.c. NaCl	10	5 hrs.	40	63.0	.....	....	.....	....	....	4	3,200 c.c.	10–15–20	.....
334.....	90	900 c.c. NaCl	10	5 hrs.	40	40.0	.....	....	.....	....	....	4	2,670 c.c.	15–20	10
324.....	100	1,000 c.c. NaCl	10	5 hrs.	45	48.1	.....	....	.....	....	....	4	2,785 c.c.	20	10–15
342.....	90	900 c.c. NaCl	10	5 hrs.	45	60.0	.....	....	.....	....	....	4	3,070 c.c.	20	10–15
343.....	85	1,285 c.c. NaCl	15	5 hrs.	45	60.0	.....	....	.....	....	....	4	3,085 c.c.	15–20	10
309.....	147	2,550 c.c. NaCl	18	6 hrs.	30	50.0	.....	....	.....	....	....	4	3,955 c.c.	10–15–20	.....
326.....	80	800 c.c. NaCl	10	6 hrs.	40	53.1	.....	....	.....	....	....	4	2,895 c.c.	20	10–15
327.....	82	820 c.c. NaCl	10	6 hrs.	40	53.1	.....	....	.....	....	....	4	3,195 c.c.	20	10–15
336.....	120	1,200 c.c. NaCl	10	6 hrs.	45	38	.....	....	.....	....	....	4	4,195 c.c.	15–20	10
277.....	225	3,650 c.c. NaCl	16	5 hrs. 7 hrs. 11 hrs.	30 30 26	55.4	1,800 c.c.	8	7 hrs.	52.9	24	7	8,715 c.c.	5–10–15	.....
282.....	232	3,350 c.c. NaCl	14½	5 hrs. 8 hrs. 6 hrs.	30 22 25	62.5	1,800 c.c.	8	5 hrs. 6 hrs.	50.0	30 40	7	8,750 c.c.	10–15	5
283.....	200	3,400 c.c. NaCl	16	5 hrs. 7½ hrs. 7½ hrs.	30 20 30	62.5	1,500 c.c.	7½	6½ hrs.	66.6	27	7	9,160 c.c.	10–15–20	.....
284.....	230	3,100 c.c. NaCl	14	6 hrs. 8 hrs. 5 hrs.	25 22 30	66.6	1,600 c.c.	7½	6 hrs.	53.3	36	7	9,100 c.c.	10–15–20	.....
285.....	228	3,400 c.c. NaCl	14½	5 hrs. 7 hrs. 7½ hrs.	30 23 30	52.7	1,850 c.c.	8	5 hrs. 7 hrs.	42.0	30 30	7	9,465 c.c.	5–10–15	.....
310.....	98	1,470 c.c. NaCl	15	6 hrs. 7½ hrs.	30 30	50.0	.....	....	.....	....	....	4	3,200 c.c.	15–20	10
303.....	170	3,000 c.c. NaCl	18	6 hrs. 6 hrs. 7½ hrs.	33 36 39	50.0	1,500 c.c.	9	6 hrs. 6 hrs.	50.0	30 40	7	8,455 c.c.	15–20	10
312.....	145	3,000 c.c. NaCl	20	6 hrs. 3 hrs.	42 48	49.1	1,500 c.c.	10	6 hrs.	68.0	40	7	6,000 c.c.	20	10–15
308.....	152	3,000 c.c. NaCl	15	7½ hrs. 7½ hrs.	40 38	73.9	.....	....	.....	....	....	4	4,435 c.c.	20	10–15

the volume of virus blood is considerably increased by the injection of salt solution.

TABLE 3.

No. of Pigs Used	Rate of Injection per Lb.	Time Let Remain	Average Percentage Increase	Value of Virus NaCl
2.....	25 c.c.	3 hrs.	183	Produced sera of low potency when used at rate of 10 c.c.–12½ c.c. per lb. of body wt. of immune.
6.....	25 c.c.	4 hrs.	147	Produced potent sera when used at rate of 10 c.c. 12½ c.c. per lb. in hyperimmunizing.
2.....	30 c.c.	4 hrs.	142	Produced potent sera when injected at rate of 10 c.c. per lb. in hyperimmunizing.
1.....	45 c.c.	4 hrs.	266	Produced sera of low potency when used at rate of 10 c.c. per lb.
10.....	30 c.c.	5 hrs.	199	Produced very potent sera when injected at rate of 10 c.c.–12 c.c.–15 c.c. per lb. in hyperimmunizing.
2.....	40 c.c.	5 hrs.	282	Produced potent sera when used at rate of 10 c.c. per lb. in hyperimmunizing.
2.....	45 c.c.	5 hrs.	306	Produced sera of low potency when used at rate of 10 c.c. per lb. in hyperimmunizing.
1.....	25 c.c.	6 hrs.	156	Produced potent sera.
2.....	30 c.c.	6 hrs.	213	Produced very potent sera when used at rate of 18 c.c. per lb. in hyperimmunizing.
1.....	33 c.c.	6 hrs.	125	Produced potent sera.
2.....	36 c.c.	6 hrs.	205	Produced potent sera when used with other virus.
3.....	40 c.c.	6 hrs.	309	Produced sera of low potency when used at rate of 10 c.c. per lb. in hyperimmunizing.
1.....	45 c.c.	6 hrs.	231	Produced serum with fair potency when used at rate of 10 c.c. per lb.
1.....	27 c.c.	6½ hrs.	255	Produced potent sera.
3.....	30 c.c.	7 hrs.	161	Produced very potent sera when used with other virus in hyperimmunizing.
1.....	34 c.c.	7 hrs.	240	Produced potent sera.
1.....	38 c.c.	7 hrs.	196	Produced potent sera when used with other virus in hyperimmunizing.
2.....	40 c.c.	7½ hrs.	455	Produced sera of low potency when used at rate of 15 c.c. per lb. in hyperimmunizing.
1.....	22 c.c.	8 hrs.	172	Produced very potent sera when used together with other virus.
1.....	24 c.c.	9 hrs.	102	Produced potent sera when used together with other virus.
1.....	26 c.c.	11 hrs.	163	Produced very potent sera.
1.....	25 c.c.	14 hrs.	36	

SUMMARY.

The virulence of salt solution recovered from abdominal cavity of virus pigs varies greatly with the amount of solution injected as well as with the time the solution remains in the cavity.

The percentage of injected solution recovered varies greatly with size and age of pig as well as with time it remains in the cavity.

Salt solution injected into abdominal cavity of virus pigs in amounts not exceeding 30 c.c. per pound of body weight and allowed to remain not less than five hours is efficient in hyperimmunizing pigs.

The use of salt solution as a virus greatly increases supply of virus and may prove a means of greatly reducing the cost of manufacturing serum.